TOPIC 33 – Pulmonary hypertension

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0356

Preventive and curative effects of anti-NGF blocking antibodies in experimental pulmonary hypertension

Véronique Freund-Michel [Orateur] (1), Christian Toussaint (2), Mathilde Dubois (1), Arnaud Courtois (1), Roger Marthan (1), Jean-Pierre Savineau (1), Bernard Muller (1)
(1) Université Bordeaux Segalen, INSERM U1045, Bordeaux Cedex, France - (2) Université Bordeaux Segalen, Bordeaux Cedex, France

Introduction: We have previously shown that expression of NGF and of its receptors is increased is both experimental and human pulmonary hypertension (PH). *In vitro/ex vivo*, we have also shown the ability of exogenous NGF to participate to inflammation, altered pulmonary vascular reactivity and remodelling, three major features in this disease. We have here studied whether administration of anti-NGF blocking antibodies *in vivo* may have preventive and/or curative effects in PH rat models.

Methods: Experimental PH in the rat was induced either by a single injection of monocrotaline (MCT, day (D)1, 60mg/kg), or after 28 days of chronic hypoxia (CH, 0.5 atm). When used as a preventive treatment, anti-NGF blocking antibodies (10µg/kg ip) were administered at D0-2-7 for MCT or at D1-8-15-22 for CH. When used as a curative treatment, antibodies were administered at D15-19-22-26 for both MCT and CH. Pulmonary arterial pressure (Pap) and Fulton index were assessed at D28. Secretion of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) from rat pulmonary arteries (PA) was assessed by ELISA. PA reactivity to prostaglandin F2α (PGF2α) or phenylephrine was assessed *ex vivo*. PA medial wall thickness was evaluated on lung sections after hematoxylin and eosin staining.

Results: In both MCT and CH rats, anti-NGF blocking antibodies displayed preventive as well as curative effects. Pap, Fulton index, cytokine secretion, hyperreactivity to phenylephrine or PGF2α and PA medial wall thickness were all significantly reduced.

Conclusion: We show here that administration of anti-NGF blocking antibodies *in vivo* can, at least partially, both prevent and reverse experimental PH in the rat. Together with our previous results observed in particular in human tissues, the present study confirms an important role of NGF in pulmonary hypertension.

0256

Improvement of right ventricular function in patients with pulmonary arterial hypertension treated with vasodilator therapy. A prospective 2D strain study

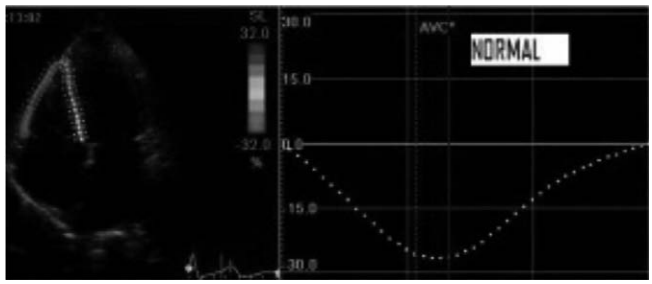
Quentin Landolff [Orateur], Baptiste Kurtz, Charlotte Vallet, Nathanaël Auquier, Dimitri Stepowski, Bertrand D'Héré, Stéphane Dominique, Hélène Eltchaninoff, Fabrice Bauer
Hôpital Charles Nicolle, Rouen, France

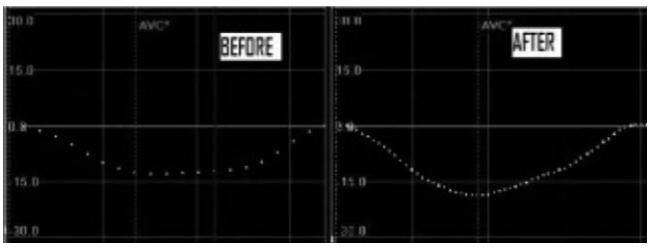
Background: Dedicated drugs improve functional status and hemodynamic in patients with pulmonary hypertension. Whether these results can be translated into an echocardiographic improvement of right ventricular function has yet to be demonstrated.

Methods and Results: 23 symptomatic patients receiving the definition of pulmonary arterial hypertension (PAH) were treated by phosphodiesterase type 5 inhibitors (26%), endothelin receptor antagonists (31%) or both (43%). Right heart catheterization and echocardiography were performed before and after 1 year of treatment for invasive measurement, right cavities area by planimetry, tricuspid anterior plan systolic excursion (TAPSE) by m-mode, longitudinal peak S velocity by tissue Doppler imaging and right ventricular longitudinal peak systolic 2D strain.

Results: There were 10 men. Mean age was 55±19 y. All were in NYHA functional class 3 or greater. Pulmonary vascular resistances (7.2±5.2 vs. 4.4±2.4 dyn•s•cm^{-5}, p=0.016) and cardiac index (3.0±0.9 vs. 3.9±1.2 l/min/m^2, p=0.003) were improved at 1 year follow-up. Echocardiographic findings after 1 year of treatment shows right ventricular cavities area reduction, increased longitudinal 2D strain (13.5±5.6 vs. 17.5±6.4, p=0.002) while longitudinal velocity (11.1±3.7 vs.11.8±3.6m/s, p=0.67) and TAPSE (16.4±3.6 vs. 19.3±6.8, p=0.07) were unchanged.

Conclusion: These results suggest 2D strain right ventricular function improvement in patients receiving oral vasodilator therapy for pulmonary arterial hypertension.





0025

Stretch-activated Channels are coupled to Ryanodine Receptors in Intrapulmonary Arterial Smooth Muscle Cells. Modifications in Pulmonary Hypertension

Guillaume Gilbert [Orateur], Jean-Pierre Savineau, Jean-François Quignard
Université Bordeaux Segalen, CRCTB - INSERM U1045, Bordeaux, France

Stretching the plasma membrane of pulmonary arterial smooth muscle cells (PASMC) provokes their contraction by a rise of intracellular calcium concentration ([Ca^{2+}]i). This mechanism called myogenic tone involves Ca^{2+} permeable stretch-activated channels (SAC) in PASMC. The aim of this study was to characterize the Ca^{2+} signaling pathways induced by a stretch of the membrane of freshly isolated PASMC from normal rats and rats with a pulmonary hypertension where the myogenic tone is stronger.

Pulmonary hypertension was induced by a chronic hypoxia of rats in a hypobaric chamber. The SAC activity was assayed by patch-clamp after a stretch of the membrane *via* a patch-clamp pipette or by an osmotic shock. Simultaneously, [Ca^{2+}]i was measured with the indo-1 probe. Finally, with a pharmacological approach and immunocytochemistry, the roles of intracellular calcium stores have been studied.

We showed that a stretch of PASMC membrane induces a rise of [Ca^{2+}]i due to a Ca^{2+} influx through SAC, amplified by two mechanisms: (1) first the Ca^{2+} entry activates K_{Ca} channels that hyperpolarize the cell and contributes to the Ca^{2+} entry by increasing its electrochemical gradient; (2) the Ca^{2+} entry through SAC is amplified by a Ca^{2+}-induced calcium-release mechanism with ryanodine receptor 1 (RyR-1) located in the sarcoplasmic reticulum (SR) under the plasma membrane. Besides, this SR is refilled by SERCA2b pumps.

In PASMC from rats with pulmonary hypertension, those phenomena are amplified with: (1) a stronger Ca^{2+} influx through SAC; (2) a more important amplification of this influx by RyR-1 and RyR-3. Furthermore, in absence of extracellular Ca^{2+}, a stretch of the membrane induces a [Ca^{2+}]i rise by an IP3-dependant pathway. All those mechanisms contribute to a stronger

[Ca^{2+} i increase and, *in fine*, in a stronger myogenic tone. This study suggests that SAC and SR calcium stores are implicated in hyper-responsiveness of the pulmonary artery that occurs in pulmonary hypertension.

0207

DHA-monoacylglyceride decreases endothelin-1 induced calcium sensitivity and proliferation in human pulmonary arteries.

Eric Rousseau [Orateur] (1), Caroline Morin (1), Samuel Fortin (2)
(1) Université de Sherbrooke, Physiologie, FMSS, Sherbrooke, Canada - (2) SCF-Pharma, Rimouski, Canada

Pulmonary artery vasoconstriction and vascular remodelling contribute to a sustained elevation of pulmonary vascular resistance and pressure in patients with pulmonary arterial hypertension (PH), an often fatal hemodynamic disease. The effect of DHA monoacylglyceride (MAG-DHA) and the role of the protein kinase C-potentiated inhibitor protein of 17 kDa (CPI-17) were determined on vasoconstriction and smooth muscle cell proliferation of human pulmonary arteries (HPA). HPA were obtained from 16 patients undergoing lung resection for carcinoma. The mechanical tension and Ca^{2+} sensitivity were measured on arterial rings treated with Endothelin-1 (ET-1) in the absence or presence of MAG-DHA. The effect of MAG-DHA on proliferation level of smooth muscle cells isolated from HPA was evaluated in order to determine the role of CPI-17 protein. MAG-DHA treatment decreased the reactivity and Ca^{2+} sensitivity induced by ET-1 in HPA. MAG-DHA treatment also decreased the expression of vascular endothelial growth factor (VEGF) induced by ET-1. Moreover, both VEGF inhibitor and MAG-DHA treatments reduced Ca^{2+} hypersensitivity induced by ET-1, which was associated to a reduction in CPI-17 and MYPT-1 phosphorylation levels. Proliferation of ET-1–stimulated human pulmonary artery smooth muscle cells (PASMc) was also decreased following CPI-17 siRNA transfection and MAG-DHA treatments. Western blot analyses revealed that MAG-DHA treatment resulted in decreased phosphorylation levels of CPI-17 and ERK in PASMc treated with ET-1.

We have demonstrated that VEGF interacted with CPI-17 signaling pathway resulting in an increase in Ca^{2+} sensitivity and proliferation of PASMc, while MAG-DHA treatment reversed these effects.

0333

Study of a new index in pulmonary arterial hypertension: the index of tricuspid displacement

Cherine Benzouid [Orateur] (1), Pierre Cerboni (2), Pamela Moceri (2)
(1) CHU Timone enfant, cardiologie pédiatrique, Marseille, France - (2) CHU Pasteur Nice, cardiologie, Nice, France

Introduction: This study intends to demonstrate the value of a new index, the index of tricuspid displacement (IDT), as measured by the ratio of ITVS at the lateral tricuspid annulus and the distance between it and the tip of the RV. The aim of this work is to define a standard for the IDT and to correlate it with prognosis parameters of PAH, as well as indices of RV function.

Method: 66 patients included: 32 cases with PAH and 34 controls. An echocardiogram was performed for each of them and a BNP's measurement within 6 hours. We precised the importance of symptoms by NYHA functional stage.

Results: The two groups are statistically comparable concerning the criteria of age, sex, BMI and left filling pressures (by E/Ea mitral). They are significantly different on the other indices. The values of the IDT are lower in cases compared with controls (respectively: 0.21 vs 0.36 ($p<0.001$). The correlation of this new index is good with TAPSE, peak of S, ITVS, and with POD (r respectively: 0.5, 0.6, 0.9, 0.65). We also note that the median of the IDT is significantly lower in patients with effusion compared to those without pericardial effusion: 0.187 vs. 0.253 ($p<0.001$). The correlation of our index, although not significant with the BNP, the Tei, SOD, the FRSVD and cardiac index (r respectively: 0.30, 0.32, 0.2, 0.34, 0.25), remains better compared to the peak of the S wave with the same parameters (r respectively: 0.19, 0.28, 0.16, 0.33, 0.26). Moreover, a threshold of IDT to 0.27 predicts a BNP>180 with good sensitivity, specificity and AUC, while a threshold of 0.19 identifies the most serious patients in stage IV of the NYHA. ROC curves show poorer results for the peak of the S wave with the same cutoff of BNP and also to predict the most serious patients with NYHA IV.

Conclusion: The index of tricuspid displacement showed encouraging results in terms of